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A PRELIMINARY ACCOUNT OF THE DEVELOPMENT OF THE APYRENE SPERMATOZOA IN *STROMBUS* AND OF THE NURSE-CELLS IN *LITTORINA*

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The dimorphic spermatozoa in *Strombus* were first described by Brock in 1887 in the case of *S. Lentiginosus*. While accurate enough in general, his account is lacking in certain interesting details and he also made no attempt to trace the developmental stages of either kind of spermatozoa Both his description and his figures, however, are sufficient to show how marked and striking is the dimorphism existing here and it is surprising that this has not been made the subject of further investigation by more recent workers.

My attention was attracted to *Strombus* in May, 1911, while at the temporary laboratory established by The Carnegie Institution of Washington at Port Royal, Jamaica, W. I. There I had the opportunity of observing the living spermatozoa and also of securing a lot of material for further study. The species studied was *S. bituberculatus*. My thanks are due to Dr. H. A. Pilsbry of The Academy of Natural Sciences of Philadelphia, who kindly identified it for me.

Adopting the terminology suggested by Waldeyer and used first by Meves ('03), the two kinds of spermatozoa found in *Strombus* are the eupyrene, *i. e.*, those that function in the ordinary way, and the apyrene whose function is unknown and in whose adult structure there is no active nuclear material. The eupyrene spermatozoa do not present any striking differences from those found in other forms which have the same sexual dimorphism, *Paludina* for example, but they lack the tenuous

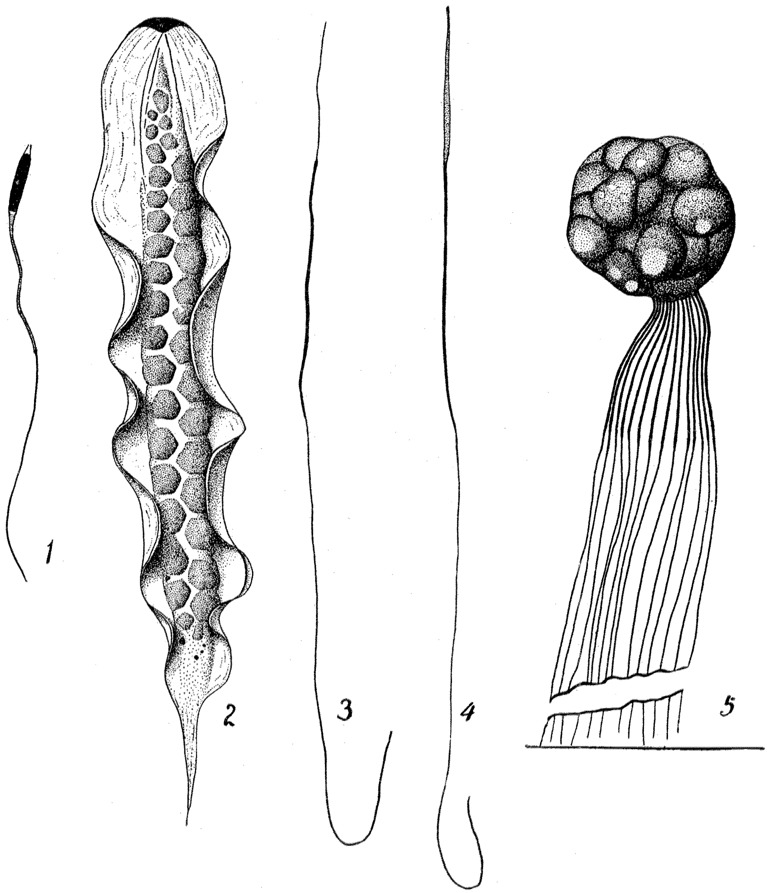
perforatorium and the very long tail-piece of the spermatozoa found in forms like *Littorina* and *Fasciolaria* (Fig. 3). The apyrene spermatozoa, on the other hand, bear very little resemblance to those described in other forms and to the eupyrene spermatozoa they bear absolutely none at all. They are very much larger than the latter and are outnumbered by them, according to Brock's estimate, about 1 to 500; the correct numerical relation existing between them in *S. bituberculatus* has not been ascertained but it is probably the same as in *S. lentiginosus*. In length the apyrene spermatozoa average somewhat over 90 micra.

The adult apyrene spermatozoön is composed of a central spindle-shaped cell body, which is long and narrow and slightly flattened dorso-ventrally, and two undulating membranes which pass down either side of the cell-body (Fig. 2). At the anterior end of the spermatozoön the membranes round out sharply to their maximum width while posteriorly they narrow more gradually and finally end in a short sharply pointed tail-piece. The interior of the cell-body is filled with a number of large polygonal bodies composed of an albumen, probably a nutritive material. These bodies are more or less regular in shape and position but they decrease in size at either end of the cell.

The living spermatozoa, as they leave the sperm-ducts, do not show any violent movements; at first long slow contraction waves pass alternately down the two membranes in a postero-anterior direction, propelling the spermatozoön in the opposite direction, that is, the posterior end is directed forward.[1] Occasionally a spermatozoön is seen moving with its anterior end directed forward. The movement of the spermatozoön is com-

[1] In *Paludina* that end of the spermatozoön which contains the remains of the nucleus, *i. e.*, the head, has been designated as the anterior end and this is the end which is directed forward in movement; it is also the end toward which the axial fibers have grown. In *Strombus*, on the other hand, there is no nuclear head in the spermatozoön and therefore, following the precedent established above, I have designated as anterior that end toward which the axial fibers have grown. It happens as a rule that in movement this end is directed backward. It was thought better to orient the spermatozoön morphologically rather than by the direction of movement. To be correct, the orientation should be reversed in both cases as the end of the eupyrene spermatozoön toward which the axial fiber has grown is the posterior one.

paratively slow and is not long continued as it soon attaches itself by means of its tail-piece to the glass slide or other object upon which it is being observed. As soon as this occurs the contraction waves pass down both the membranes simultaneously



FIGS. 1 to 5. Initial magnification of 1,850 diameters, reduced one third. Fig. 1, eupyrene spermatozoön of *Strombus bituberculatus*. Fig. 2, apyrene spermatozoön of the same form. Fig. 3, spermatozoön of *Littorina nebulosa* showing the long, thin perforatorium. Fig. 4, spermatozoön of *L. angulifera* showing the perforatorium swollen after being in sea-water for some time. Fig. 5, nurse-cell of *L. nebulosa* with attached spermatozoa; drawing made from a living cell.

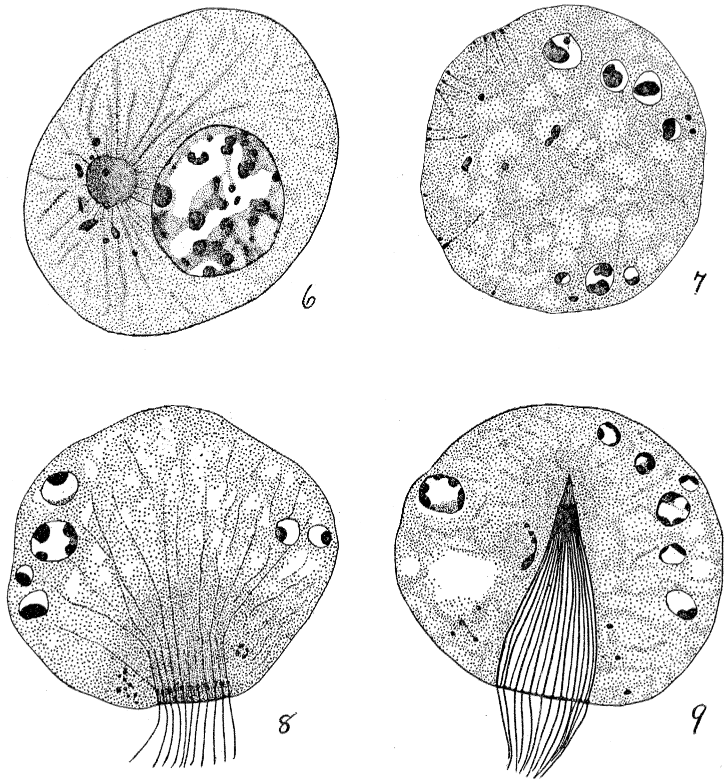
and they become much shorter and faster. With the spermatozoön attached in this way, the membranes may continue to be active for an hour or two. It very frequently happens, however,

that the tail-piece breaks off and the spermatozoön swims away with a much more rapid movement than it had at first. When the tail-piece begins to break off it can be seen to be composed of a number of fused flagella; sometimes as the spermatozoön moves away one or more of the flagella may be seen still adhering to it. This explains the statement of Brock to the effect that a tuft of flagella, which is invisible at first, is to be seen after the spermatozoön has been swimming about for a while. It very frequently happens that long before the undulations of the membranes have ceased, the spermatozoön flattens out and the albuminous bodies break down, leaving in their place a brownish, semi-fluid substance in which, however, may still be seen the outlines of those bodies.

As in *Paludina*, the apyrene spermatocytes of *Strombus* are easily recognized. They are large pear-shaped cells provided with a nucleus of regular pattern and a large centrosome about which may be seen an inner clear court and an outer dark court. Until a very late stage in their growth period they retain a connection with the cyst-wall of the testis by means of a short stalk; later they lose this attachment and become spherical. By this time the chromatin has begun to form in lumps beneath the nuclear membrane and from a large number of centrioles lying at the periphery of the centrosome strong radiations may be seen to pass out in all directions (Fig. 6). In the outer court, but away from the nucleus, lies a mass of mitochondria. A division of the nucleus and cell never follows; instead, the nuclear wall breaks down and the centrosome with its radiations disappears. A little later the chromatic masses are seen scattered through the cell while the centrioles have moved to the periphery of one half of the cell where they are easily recognized by their radiations (Fig. 7). The cell now begins to develop directly into the spermatozoön.

The chromatin, as such, takes no further active part in the development of the spermatozoön; the fragments very soon begin to become vesiculated and to degenerate. The centrioles mass together at a point just beneath the cell membrane where they divide (Fig. 8). One half of the number of daughter or secondary centrioles remain attached to the cell-membrane and

from them grow out flagella which ultimately fuse to form the tail-piece. The others move across the cell forming a bundle of axial fibers. At its base the bundle is round but it becomes more and more flatly oval as the fibers move across the cell (Fig. 9).



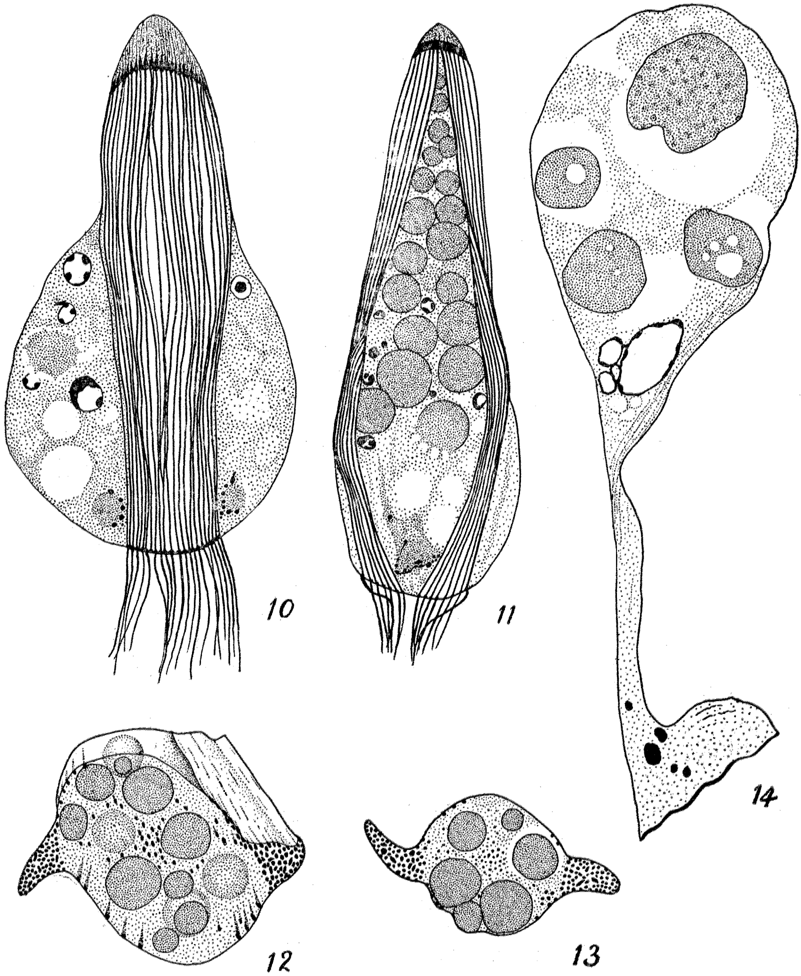
FIGS. 6 to 9. Initial magnification of 3,450 diameters, reduced one third. Various stages in the early development of the apyrene spermatozoa of *Strombus bituberculatus.* Fig. 6 is a spermatocytic stage just before the breaking down of the nucleus and the disappearance of the centrosome. Fig. 7 is a stage following the breaking down of the nucleus; the centrioles figured represent only a portion of the total number in the cell. Fig. 8 shows the beginning of the bundle of axial fibers. Fig. 9 shows the fibers grown partly across the cell; the view is taken at an angle of 90 degrees from that of Figs. 8 and 10.

The mitochondria may be seen to form the inner margin of a ring of differentiated cytoplasm which surrounds the base of the bundle. They persist here until the spermatozoön has reached its adult form (Figs. 8 and 10).

When the bundle of axial fibers, by its growth, has increased the length of the cell by about one third, it splits and the halves begin to move to either side of the cell (Figs. 10 and 11). This movement is caused by a twisting of the secondary bundles, due probably to the unequal growth of the fibers. This process continues until they push out the cell membrane on either side to form the undulating membranes (Figs. 12 and 13). Fig. 12 is an oblique section through the middle of a spermatozoön which has not quite matured; later the membranes become flatter and wider as shown in Fig. 13, which is a more anterior section of a still older spermatozoön. As is indicated in Fig. 12, the continued growth of the fibers without a compensating increase in the length of the cell causes several even folds to occur throughout the length of the membranes. Some of the axial fibers do not participate in the formation of the membrane. As the bundles begin to evaginate the cell membrane, a few of the fibers begin to migrate and finally come to lie longitudinally across the cell and just beneath its membrane (Figs. 12 and 13).

The albuminous bodies are formed in the same way as Kuschakewitsch ('11) has briefly described in *Vermetus gigas*. Large vacuoles appear in the cytoplasm, first in the anterior portion of the cell, and these gradually become filled with an albuminous substance. When such a vacuole has been almost filled but before a membrane is formed, narrow strands may be seen connecting the albumen with the surrounding cytoplasm (Fig. 11). The cytoplasm which is not displaced by the formation of these bodies becomes fibrillar, the greater part of it forming a core down the center of the spermatozoön (Figs. 12 and 13).

As the spermatozoön develops, the vesiculated nuclear fragments which were scattered throughout the cell continue to degenerate. They gradually become more condensed and darkly staining and undergo further fragmentation. They may dissolve *in situ*, but they may also go to form the many small granules that lie in amongst the fibers composing the undulating membranes (Fig. 13). These granules are probably mitochondria and while at present it cannot be definitely asserted that they are thus of a direct nuclear origin, there is considerable evidence in favor of this view. The mitochondria which originally sur-



FIGS. 10 to 14. Initial magnification of 3,450 diameters, reduced one third. Figs. 10 to 13, various stages in the later development of the apyrene spermatozoa of *Strombus bituberculatus*. Fig. 10, bundle of axial fibers beginning to split; a few albuminous bodies, not figured, have been formed in the anterior portion of the cell. Fig. 11 shows the completed splitting of the bundle of axial fibers; the cell is not cut through its greatest breadth. Figs. 12 and 13 are sections through two nearly adult spermatozoa. Fig. 14 is a nurse-cell of *Littorina nebulosa* still attached to the wall of the testis; the stalk was about to be severed just below the degenerating nucleus.

rounded the centrosome remain at the posterior end of the spermatozoön.

The species of *Littorina* in which have been found a free nurse-cell to which the spermatozoa are attached are *L. angulifera*, *L. nebulosa* and *L. rudis*. The first two species were studied at Port Royal, Jamaica, along with *Strombus bituberculatus*, and they too were identified by Dr. Pilsbry.

If the sperm-ducts of *L. nebulosa* or *L. rudis* are ruptured, the contents, when diluted with sea-water, will appear under the microscope as a great number of spheres to each of which is attached a tuft of spermatozoa. The spheres are nurse-cells composed of vacuolated yolk bodies and a degenerate nucleus (Fig. 5). The nurse-cells of *L. angulifera* differ from those of the other two species in that here the yolk bodies are not vacuolated and they partly enclose a long thick cytoplasmic rod to one end of which are attached the spermatozoa. In all three species only the perforatoria and possibly the tips of the heads of the spermatozoa are inserted into the cell.

The first movement to be seen is a rhythmical and uniform beating of the tuft of spermatozoa which sends the nurse-cell rapidly forward. Later the spermatozoa beat independently and the tuft spreads. Very frequently the spermatozoa of one nurse-cell become entangled with those of another; in that event the nurse-cells are drawn together and held by an agglutinous substance forming the pabulum in the cell into which the spermatozoa are inserted. In this way a great many nurse-cells are drawn together and from such a mass the spermatozoa may later be seen protruding on all sides and beating regularly like cilia. This beating of the spermatozoa will continue for several hours.

In case a nurse-cell has not become entangled with others, the spermatozoa soon free themselves; their heads become further and further separated from the nurse-cell until the attachment is completely lost. A sperm thus freed is seen to have a long, thin perforatorium behind which is the head; the latter passes almost imperceptibly into a very long tail (Fig. 3). After the spermatozoön has been swimming about in the water for a time the perforatorium becomes swollen; this is what has usually been figured as the sperm head. The whole process can be seen to better advantage in *Fasciolaria*.

The nurse-cells develop from large cells which are attached to the walls of the testis by a long stalk. These cells closely resemble the apyrene spermatocytes of *Strombus* except that they lack the pronounced centrosome of the latter. The only indication of such a structure in the case of *L. nebulosa* is a series of fibers running up one side of the cell. This later disappears and probably forms the portion of the cell to which the spermatozoa are attached (Fig. 14). In *L. angulifera*, in the early stages of the nurse-cell, there is a darkly staining body lying in the cytoplasm which grows to form the rod spoken of above; this also may be of a centrosomal origin. The formation of the yolk bodies is much the same as that of the bodies described in *Strombus*. They differ, however, in that here they reach a much larger size and then fragment into two or more parts. Coincident with this cytoplasmic differentiation the nucleus undergoes partial degeneration; it simply becomes more and more vacuolated but never completely disappears.

Before the nurse-cell has reached its full development it loses its connection with the wall and moves into the lumen of the testis. Here the spermatozoa become attached. The nurse-cells function as such in the sperm-ducts. Sections of *L. rudis*, made from a specimen killed in February, showed the nurse-cells in the sperm-ducts to be in a more or less depleted condition.

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